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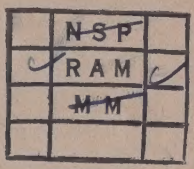

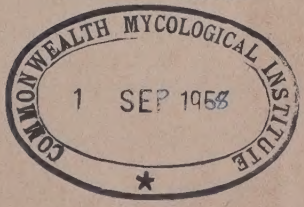

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Contents

Factors in propagating presumably virus-free Prunus *understock clones by softwood cuttings*

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Introduction

For critical investigations, stone fruit virologists have an urgent need for large numbers of *Prunus* spp. clones free from viruses. Such a want has led to the present research studies.

Clones free from the "ring spot virus complex" are one of the most pressing needs because one or more entities of this complex is usually associated with the more serious stone fruit virus disorders. The current premise is that symptoms produced on a known understock clone by a given virus or virus complex will be constant. Thus, using specific inoculated members of a particular understock clone, a differential series can be established. Varieties budded to these differential ramets can be evaluated in regard to susceptibility, resistance, or tolerance to given virus components. Also, workers can screen selections for the presence of a particular virus strain or complex on the same basis.

Yield reductions owing to known virus strains can be studied without the error associated with differing rootstocks, such error being due in part to virus passage through seeds, genetic disorders, and compatibility. In addition, from a horticultural standpoint, selected clones would remove the error of understock variability. Thus, variety comparisons of yield, rootstock effects, fruit characteristics, etc. would be more accurate.

The ability of different rootstocks to furnish comparable nutrient materials to a given scion has not been given much attention. Such a study is needed and clonal rootstocks must be used for this kind of study. It is known that *Prunus* rootstocks differ in relative fungal and bacterial disease resistance. Vegetative propagation of selections resistant to these pathogens also would be advantageous.

Some of the difficulties encountered in the vegetative propagation of *Prunus* spp. require special consideration. This genus has certain peculiarities when compared with many other kinds of woody plants. *Prunus* spp. leaves transpire more rapidly than most woody plants (Comar and Barr, 19).[1] They also produce mucilaginous materials at cut surfaces. Such materials partially block the conductive tissues of excised stems in a few days. These are primary factors in desiccation, and hence death of cuttings.

It is, therefore, necessary to reduce water losses to a minimum without injuring the cutting from the time it is made until it roots. Also, early rooting must be promoted to avoid complications induced by plugging of xylem vessels. To promote rooting one must maintain adequate photosynthesis and supply sufficient nutrients to provide a threshold above respiratory requirements. Only in this way can cuttings manufacture the materials needed to produce a functionable root system.

Thus, the physiologic factors for successful vegetative propagation of *Prunus* spp. lie in two categories:

1. Environmental control of the propagating chamber.

[1] Numbers in parenthesis refer to *References*, page 31.

2. The physiologic conditions inherent in, or imposed upon the cuttings both before and during the period of root formation.

Study objectives. The present study was made to determine and evaluate those critical environmental and physiological factors necessary for successful vegetative propagation of ring-spot-virus-free *Prunus* spp. understocks.

Materials and Methods

Selection of presumably ring-spot-virus-free material for propagation

In this study, means to propagate cuttings of *Prunus* spp. understocks used in the nursery industry of Washington were emphasized. Species investigated were:

1. *Prunus mahaleb* L.,
2. *Prunus avium* L.,
3. *Prunus persica* Sieb. and Zucc.,
4. *Prunus cerasifera* Ehrh. var. *myrobalan* L.

Material to be used for establishing understock clones cannot be chosen at random. Cation (11) showed that ring spot virus will pass through seeds of *P. mahaleb.* Cochran (16, 17) demonstrated its passage through seeds of *P. avium* and *P. persica.*

The presence of the virus in trees of *Prunus* spp. is not always detectable by observations alone (Milbrath and Zeller, 41). Some strains of the complex that are apparent in, and destructive to one species may be present but "latent"[2] in another. In certain cases temperature and season influence symptom expression and symptoms may not be observed (Keitt and Moore, 35).

Nearly all of the more serious virus diseases of stone fruits are associated with ring spot virus and/or its complexes (Blodgett, 6). The various strains of ring spot virus and its complexes can be determined in *Prunus* spp. in several ways. Detection methods can be placed under two main categories, chemical tests or indicator plants. At the present degree of development, indicator plants (Blodgett, 6; Fink, 22; Milbrath and Zeller, 40, 41; Moore and Keitt, 43; Moore *et al.,* 42) appear to be much more reliable than chemical tests (Lindner, 36; Lindner *et al.,* 37; Simonds and Bodine, 54). Indicator plants as now employed may be certain varieties of various *Prunus* spp., or certain members of the family Cucurbitaceae.

The most common method for indexing *Prunus* spp. trees is to bud healthy, indicator trees of a susceptible variety with buds from a test plant. Moore and Keitt (43), using healthy Montmorency cherries, determined the presence of "latent," necrotic ring spot virus in sour cherry. Fink (22) detected ring spot virus in cherries by indexing selections on *Prunus tomentosa* Thumb. seedlings. Blodgett (6) demonstrated the ring spot virus in several *Prunus* spp. by indexing selections on *P. persica* Sieb. and Zucc. var. *Lovell* seedlings.

The most efficient and demonstrative index plants for detecting "latent" ring spot virus are two flowering cherry varieties selected by Milbrath and Zeller (40, 41). These are *Prunus serrulata* Lindl. var. *Shirofugen* and *P. serrulata* Lindl. var. *Kwanzan.* These two flowering cherry varieties in combination indices are considered sensitive to all serious as

[2] "Latent" viruses are those entities present which produce no symptoms for one reason or another. (See Milbrath and Zeller 40, 41.)

well as "latent" stone fruit ring spot virus strains from all known sources of *Prunus* spp. trees.

Another index plant believed by some to be as critical or better than the flowering cherries, is the cucurbit, National Pickling Long Green cucumber. The techniques used involve considerable greenhouse culture, timing, and mechanical inoculation. The use of this index plant has been developed by Willison (64), Willison and Weintraub (65, 66), and Weintraub and Willison (62).

Because of their efficacy in determining the presence of ring spot virus strains and the ease of handling and timing, the Lovell peach index of Blodgett (6) and the flowering cherry index of Milbrath and Zeller (40, 41) were used in these investigations to select presumably virus-free material for propagation.

Lovell peach indexing

The Lovell peach index consisted of plate-budding each of three, one-year-old dormant seedlings in March with a bud from each of three budsticks of the individual being tested. After inoculated trees were stored for one month to callus, they were lined out. An uninoculated "check-tree" was placed between each index group. After every 10 indices, 5 additional uninoculated "check-trees" were planted.

When growth was well started, trees were cut off at the soil level or just above the upper test bud. All but one, strong, sucker-shoot were removed. New suckers were kept stripped-off until readings were made in July .

The index was evaluated on vigor of growth of inoculated seedlings compared with adjacent "checks" (figure 1). The

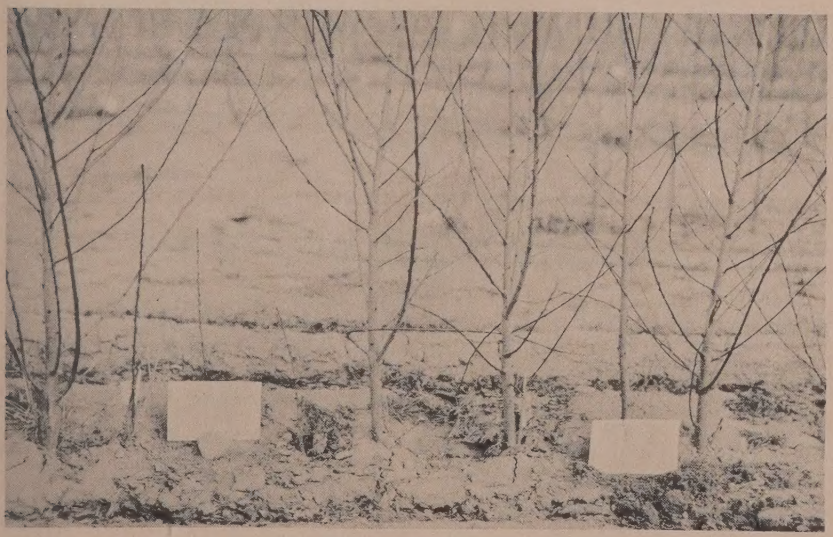

Fig. 1. The Lovell peach seedling index method for exposing "latent" virus in stone fruits. From left to right: an unbudded check seedling, three seedlings budded with a selection containing "latent" virus, another unbudded check seedling, and three seedlings budded with a selection apparently free from "latent" virus infection.

measurement used was the caliper of the tree in millimeters divided by tree height.

A shock effect owing to the presence of a ring spot virus strain or complex transmitted from test buds dwarfed the growth of the Lovell peach seedlings in varying degrees. Source indices that did not measurably dwarf Lovell peach were selected for initial propagation tests. These sources were re-indexed on the flowering cherry indicator plants.

Flowering cherry indexing

The flowering cherry indicator plants produce a localized reaction to the presence of ring spot virus, a systemic reaction, or both. The local reaction consists of necrosis of index plant tissue beneath and adjacent to the site of bud insertion, and this is usually accompanied by considerable gumming. Such a reaction is generally associated with more virulent strains of the ring spot virus complex on Shirofugen. The systemic reaction is manifested by a twisting and curling of leaves, combined with malformation.

The systemic reaction is not apparent until new buds on an inoculated index plant break dormancy and leaves expand. Therefore, selections to be tested must be indexed in the fall before leaf fall. Readings must be made the following spring after growth has started.

In practice, individuals to be indexed on flowering cherries were selected if there were no apparent virus symptoms throughout the growing season and indices were negative on Lovell peach. Then in early fall three bud-sticks of current season growth were removed from different locations on the tree to be tested. One bud from each bud-stick was inserted into a current season branch of the index tree (figure 2A). Readings on local reaction were made early the next spring before the index plant leafed out. Later readings were made for any systemic expression of other strains of the ring spot virus complex. If inserted buds had formed a union with the index plant and no local reaction had occurred (figure 2B), or if no systemic reaction had occurred, the source tree was tagged for use.

Selection of materials and variables

Type of cuttings and age of shoots

Considerable variation among species exists in regard to the kind of cutting best suited for vegetative propagation of different woody plants. Stem cuttings are usually described as "softwood" or "hardwood" (Avery and Johnson, 2). "Softwood" cuttings may be "green" (taken from new shoots that have just attained full growth), or "half-ripened" (taken from branches or stems that have ceased growth for the season, but retain their leaves for some weeks). "Hardwood" cuttings are those taken from leafless (dormant and ripened wood) plants.

The rooting of "softwood" or "hardwood" cuttings is influenced by:

1. Position of the cut with respect to buds.
2. Presence of an apical bud.
3. Length of cutting.
4. Type of wood.
5. Slope and smoothness of the cut at the base of the cuttings.

In addition, Adriance and Brison (1) state that rooting response of many species is affected by:

1. Cuts made slightly above the base of current season growth.

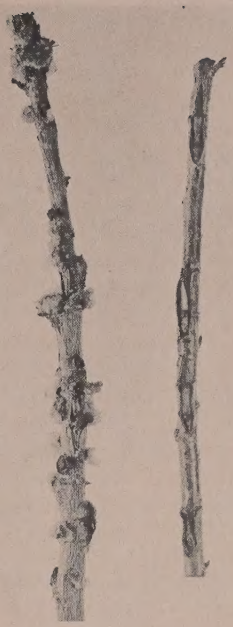

Fig. 2 A. Fig. 2 B.

The Shirofugen flowering cherry index method for exposing the presence of "latent" virus. 2A shows an index plant recently budded with a selection under test.

2B, left, shows a positive reaction of the host plant to the presence of "latent" virus in the selection budded to it. 2B, right, illustrates a negative reaction of the host plant, indicating the absence of "latent" virus.

2. Cuts made at the base of current season growth.
3. Including a mallet of one-year-old wood.

Preliminary tests to determine the best propagating material for rooting *Prunus* spp. resulted in selection of the "softwood" kind (either "green" or "half-ripened"). Other tests showed that maturity of growth and the presence of an apical bud had a variable effect on rooting when no growth regulator was used. However, no differences were noted in these respects when cuttings were treated with growth regulators of the indole series.

Further studies determined that excision with a sharp knife just below a bud markedly increased rooting of certain individuals, while four to six inch cuttings rooted better than any other length of cutting tested. Therefore, "half-ripened" cuttings without an apical bud, having one or two leaves plus a bud at the basal end were used in most of the detailed studies. In other studies, similar type cuttings having an apical bud were used.

Rooting medium

The medium and the type of watering best suited for propagating softwood cuttings depends upon the species being propagated (Chadwick, 12, 13). As a general rule, mixtures of peat moss and sand afford unusually good means of obtaining widely divergent conditions of pH, aeration and moisture holding capacity that may affect a species' response (Kains and McQuesten, 34).

In a series of preliminary tests, quartz sand, pit sand, "desert" or lava sand (as found near Moxee, Washington), Vermiculite, Canadian (British Columbia) peat moss, German peat moss, *Sphagnum* moss, sawdust, crushed basalt and very fine granite sand were tried alone and in various combinations. The best medium for rooting of *Prunus* spp. cuttings was found to be one with adequate water holding capacity, good drainage, and efficient aeration.

Such a medium consisted of any type of sand that would pass through one-eighth-inch hardware cloth but not through one-sixteenth-inch mesh screen, plus Canadian peat moss at the ratio of two parts sand to one part peat moss. This kind of rooting medium was used in all the detailed studies to follow.

Growth regulators

Indolebutyric acid (IBA) was the most satisfactory of the growth regulators tested for propagating *Prunus* spp. Preliminary trials were made with several regulators, including indoleacetic; naphthaleneacetic; indolebutyric; indolepropionic; 2, 4, 5-trichlorophenoxyacetic acids; and potassium naphthaleneacetamide. All growth regulators were tested in concentrations from 10 ppm. to 8000 ppm. in various carrier materials.

The growth regulator carriers tested were lanolin, Fermate, Parzate, talc, 50 per cent ethyl alcohol, and distilled water. Talc afforded the best carrier qualities. Indolebutyric acid at a concentration of 3000 ppm. in talc was selected as the most satisfactory formulation in these preliminary tests. Since the Merck and Company product, Hormodin No. 2 powder, is this same formulation, it was used as the growth regulator in detailed experiments as a standardized preparation.

Nutrients and other materials

Yellowing and defoliation usually occur in the cutting bed about ten days after *Prunus* spp. cuttings are made. On the assumption that low carbohydrate and nitrogen levels within cuttings contributed to these symptoms, carbohydrate and nitrogenous materials plus certain metabolites were administered in preliminary tests to hasten rooting.

Sucrose was selected as the best carbohydrate source. Glucose was equally good, but sucrose was cheaper and also reduced injury from foliar applications of nitrogen.

Urea was the best source of nitrogen in trials comparing foliar applications of ammonium hydroxide, ammonium phosphate, ammonium sulphate, urea, asparagine, and glutamine.

Thiamine and nicotinic acid combined and alone in foliar applications as metabolites hastened rooting the most in trials comparing vitamin B complex, brewer's yeast, thiamine, nicotinic acid and pyridoxine in various combinations.

Preliminary results suggesting application of sucrose, urea, thiamine and nicotinic acid as a nutrient, foliar treatment are shown in figure 3.

Application of adenine to cuttings as a metabolite was investigated for several purposes:

1. To exclude its deficiency as a possible limiting factor in phosphate energy (Skoog, 55; Bonner, 7, 8).

2. To resolve its efficiency on breaking of bud dormancy (Bennett and Skoog, 5; Skoog *et al.*, 58).
3. To augment the thesis, if true, that there is a balance between adenine and auxin in regard to differentiation (Skoog and Tsui, 56, 57).

Preliminary trial results were inconsistent in the response of species to treatment with adenine. The mode of application also induced variable results. In the experiments to follow, therefore, this metabolite was incorporated as an undetermined variable.

Sucrose, urea, thiamine, nicotinic acid and adenine, alone and in various combinations as foliar dips, were selected for further study as a nutrient metabolite supply to prevent chlorosis and defoliation and to determine their effects on rooting and breaking of bud dormancy.

Light

It is often necessary to reduce solar light intensity to control temperature, although softwood cuttings root best when light intensity is high. Correlation of photosynthesis with various environmental factors is reviewed in detail by Rabinowitch (51, 52, 53), Granick (25), Gaffron and Fager (23) and Brown and Frenkel (10). Since light as a controlled variable is important in standardizing physiological responses, selection of a substitute for solar insolation was necessary in order to obtain high light intensity with minimal radiant heat.

Withrow and Withrow (68) compared the effect on growth of radiation from incandescent filament, fluorescent, and mercury-arc sources of light. With equal intensity, certain plants responded better to incandescent filament sources and others better to fluorescent sources. Parker and Borthwick (49), Naylor and Gerner (44), Hartmann and McKinnon (31), and Hamner (30) reported details on construction of fluorescent light installations. Parker and Borthwick used slimline, 4500°, cool white, fluorescent lamps supplemented by incandescent filament bulbs with excellent results on all plants tested. They were able to control temperatures without difficulty.

Thus, to limit error from heat transfer, fluorescent lamps supplemented by incandescent filament bulbs were used as a high light intensity source in these propagation experiments.

Temperature

The temperature coefficient for cell growth and elongation is high (Chao and Loomis, 15). The optimal temperature for the growth of plant parts usually lies near 25°C. (77°F.), (Went, 63). Therefore, an air temperature range of 70°F. to 80°F. was selected for these studies.

Dormancy

Breaking of bud dormancy is one of the critical factors in establishing rooted softwood cuttings of *Prunus* spp. Root initiation must take place before buds break dormancy. Dormancy must be broken after root initiation or cuttings generally die.

Plants respond to cyclic variations of temperature. Went (1953) suggests that obscure biochemical properties within plants are affected by annual cyclic changes in temperature. He considers this phenomenon the basis for abscission and dormancy in deciduous plants. The average number of hours below 45°F. (7.2°C.) required to break dormancy of peach buds has been shown to be approximately 1000 hours (Weinberger 61), Yarnell (69). Chandler, *et al.* (14) reported that a calculated 1440 hour period below 45°F. was necessary to break dormancy of cherry buds. The period need not be continuous, however.

Since a temperature of 45°F. or lower in the cutting chamber is untenable, a biochemical basis for breaking bud dormancy is suggested. The data of Guthrie (26), Hemberg (32, 33), and Michener (39) indicate the biochemical basis for breaking bud dormancy is a mechanism of starch hydrolysis at low temperatures, or removal of inhibitors from the buds or branches. Guthrie (27) and Bennett and Skoog (5) suggest that dormancy is caused by development of growth-promoting substances in buds, Skoog and Tsui (56, 57) point out a ratio between growth regulator and adenine.

Chemicals have been used to break dormancy in bearing stone fruit trees. Guthrie (28) broke dormancy of peach buds four months ahead of control trees. He sprayed trees with one per cent solution of chloro-o-phenylphenol in a light petroleum oil-penetrol emulsion. By injecting 5 mg. of glutathione, Guthrie (27) had previously reduced the dormancy period twenty days. He later found that a yeast extract was even better than glutathione (29). His results suggest that Vitamin B is the critical factor. Bennett, *et al.* (4) also found that a yeast extract was more active than glutathione.

Since Vitamin B_1 and nicotinic acid of the Vitamin B complex, as well as adenine, were to be included in the nutrient portion of the experiment, dormancy breaking by chemicals was considered measurable in a factorial experiment.

Humidity

High relative humidty must be maintained in the cutting bed to prevent desiccation of cuttings before they root. Generally, the walls of the propagating frame are sprinkled and the cutting foliage is syringed lightly with water (Adriance and Brison, 1). However, such practices provide ideal conditions for growth and spread of micro-organisms. Careful sanitation in the cutting bed is essential.

Water loss via transpiration is normally through the stomata of the leaf and is, of course, the predominant form of water loss from leafy plants.

Bonner and Galston (9) show light, temperature and relative humidity as the primary environmental factors which control transpiration. A chain effect is started through illumination, particularly solar, which:

1. raises the temperature at the leaf surface, bringing about a diffusion gradient between the leaf and the surrounding atmosphere, or
2. initiates photosynthesis so that carbon dioxide in the substomatal cavity is removed. The concentration of carbon dioxide in the substomatal cavity is believed to control the opening and closing of stomates. When the carbon dioxide content of substomatal cavities is low, stomata open.

Thus it is obvious why high relative humidity must be maintained. However, excised portions of a plant, such as cuttings, dry out even though high relative humidities are maintained.

As the water content of the cutting leaves and stem tissues diminishes, water loss through the cuticle becomes more critical to water conservation (Crafts, *et al.*, 20). The thickness and the degree of permeability of the cuticle, although unimportant when active stomatal transpiration occurs, are significant when leaves are under stress for water.

Cuticular transpiration and antitranspirants

The thickness and composition of the cuticle varies with the species, age of plant, position of the leaf, and the physiological condition of the plant (Crafts, *et al.*, 20). Nigam (48) found that little cuticle was formed under very humid

conditions. Plants changed from a humid environment to outdoor conditions under direct sunlight were injured or killed in a few hours. Lack of cuticle production and anaesthetized stomata induced by the extra humid conditions were considered responsible.

Pieniazek (50) showed that natural waxy deposits on the surface of the leaf greatly inhibited water loss. Based on the work of Neilson (46, 47), Maney (38), and Tukey and Brase (59), waxes, paraffin and oil emulsions have been used to inhibit transpiration from bearing cherry trees. Bennett (3) and Wilson (67) developed satisfactory emulsifying agents for paraffin wax. These materials have been used in various fields of horticulture to check water loss, but have not been employed in propagation tests.

Thus, in preliminary propagation experiments emulsifiable wax materials combined with a high relative humidity in the cutting bed were tested for a means to reduce cuticular transpiration, and hence prevent desiccation of cuttings.

Of the several emulsifiable materials tested, the one suggested by Neal, *et al.* (45), showed the greatest promise (figure 3). This formulation is the same as a commercial preparation, Dowax 222, of the Dow Chemical Company. This antitranspirant extended the time of leaf abcission well beyond three weeks from the date that cuttings were made.

Fig. 3A.

Fig. 3B.

Response of *Prunus mahaleb* cuttings to treatment with nutrients, antitranspirant, or both. 3A, left: defoliated cuttings three weeks after treatment with nutrients but without antitranspirant. 3A, right: healthy cuttings three weeks after treatment with nutrients and antitranspirant. 3B shows cuttings three weeks after treatment with antitranspirant but without nutrients.

The formulation of this emulsifiable wax follows:

Material	*Percentage by weight*
Paraffin wax (Parawax)	27
Linseed oil, body W	11
Span, No. 40 (emulsifying agent)	5
Tween, No. 40 (emulsifying agent)	4
Bentonite	1
Water	52
	100

This particular wax emulsion preparation at a 1 to 99 dilution reduced transpiration of growing trees for one month when applied in three sprays by Neal, *et al.* (45). At a dilution of 1 part wax emulsion to 10 parts water, Neal reported that transpiration was reduced 52 per cent, up through the sixth day and remained at this level through the eleventh day. Injury was noted only after temperatures above 100°F. had occurred.

Many propagators prune leaves to prevent desiccation, using the principle of reduced leaf surface for water economy in cuttings (Kains and McQuesten, 34). The close correlation between rooting response and photosynthesis in difficult-to-root species must be taken into account, however, before such practice is deemed beneficial. Therefore, no leaves were pruned on cuttings in these studies.

Control of standard conditions and treatments selected

Mechanical control of environment

To control the environment, a propagating frame was constructed so that light, temperature and humidity could be regulated. Cool-white, 4500°, slimline fluorescent lamps were combined with incandescent bulbs for illumination. Air temperature was controlled by water evaporation through fog mist nozzles. The fog mist also kept the relative humidity high in the cutting bed.

The propagation frame was constructed from a surplus army bunk-bed. The springs were removed and the breastwork of the upper bunk was inverted as a mount for the fluorescent lamps. The holes for the coil springs, spaced at two-inch intervals, were just right for mounting lamp holders.

Eighteen, 72-inch, T-8, 4500°, cool-white, fluorescent lamps were mounted on the frame. Nine 300 ma. capacity ballasts for the lamps were mounted on two-by-fours placed across the inverted spring support to allow ventilation.

Twelve inches were left between the ballast rack and the top of the fluorescent lamps. In this space two 3 foot by 4 foot pieces of 32 gauge aluminum sheeting were mounted as reflectors. This reflector was painted white. The ends were left open for heat-escape.

Two 100 watt, incandescent light bulbs were placed on the reflector to augment red and yellow portions of the spectrum. Over 4000 foot candles were obtained one foot below the fluorescent lamps through double strength glass. A time switch controlled the lights so that length of day could be regulated (figure 4).

The upper surface of the propagating chamber was placed one foot below the fluorescent lamps on top of the lower bunk frame. Seven-foot angle irons were bolted to the ends of the lower bunk frame to serve as runners on which to slide the chamber from under the light source for accessibility (figure 5). The internal dimensions of the chamber were one foot by three feet by six feet.

The lid was made from a conventional propagating-frame cover from which all the small panes and wooden cross sup-

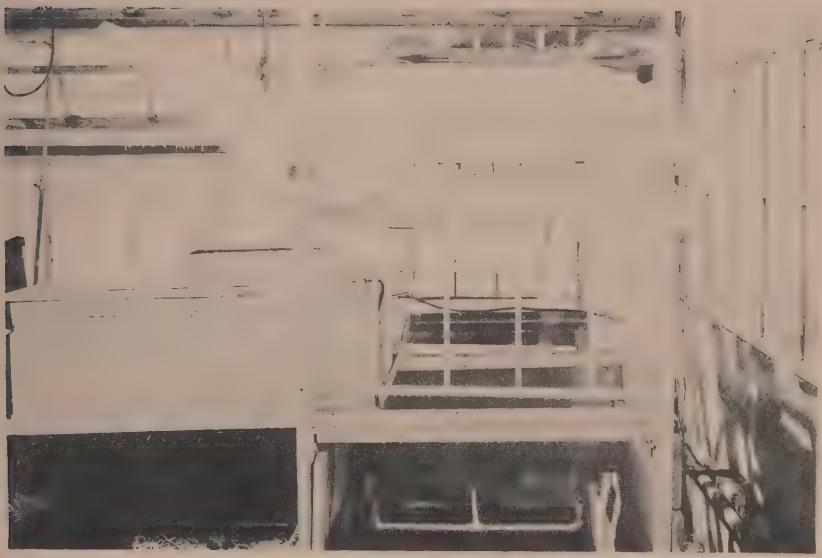

Fig. 4. End view of controlled environment apparatus with burlap-covered frame removed to show arrangement of fog-mist nozzles beneath propagation chamber.

Fig. 5. Controlled environment apparatus with propagating chamber pulled forward and cover raised to show means of access and vent arrangements for circulation. Humid air is drawn in from ends and exhausted through center. Note humidostat in center rear for humidity control through regulation of fog mist nozzles underneath.

ports had been removed. In place of the small panes, two 32 by 34 inch, double strength, glass panes were mounted to eliminate shadows (figure 5).

The floor of the chamber was constructed with three-inch vents at both ends and a four-inch vent in the center (figure 5).

Below the central vent, in the area beneath the movable chamber, a 1 by 1 by 3 foot box was constructed. This box was left open at the top. At the rear a hole was made to which a 50 c.f.m., Falco squirrel-cage exhaust fan was attached (figure 6). In operation this fan drew air into the closed chamber from both ends and exhausted it through the center vent. The fan could change the chamber air just under four times per minute when the chamber was filled with cuttings in flats.

The front and rear sides of the frame below the propagating chamber were enclosed. Air pulled into the lower area of the construction from the ends was drawn through burlap covered forms. In this lower area, two fog-mist nozzles of two-gallons per-hour capacity were mounted at each end of the enclosure with their mist-streams directed toward the burlap forms and the vents (figure 4).

Nozzles were controlled by a solenoid valve located on the water line leading into the chamber from the rear, below the exhaust box construction (figure 6). The solenoid valve was regulated by a humidostat at the center rear of the propagating chamber, above the center vent (figure 5). The exhaust fan was controlled by this same humidostat.

A diaphragm type temperature control

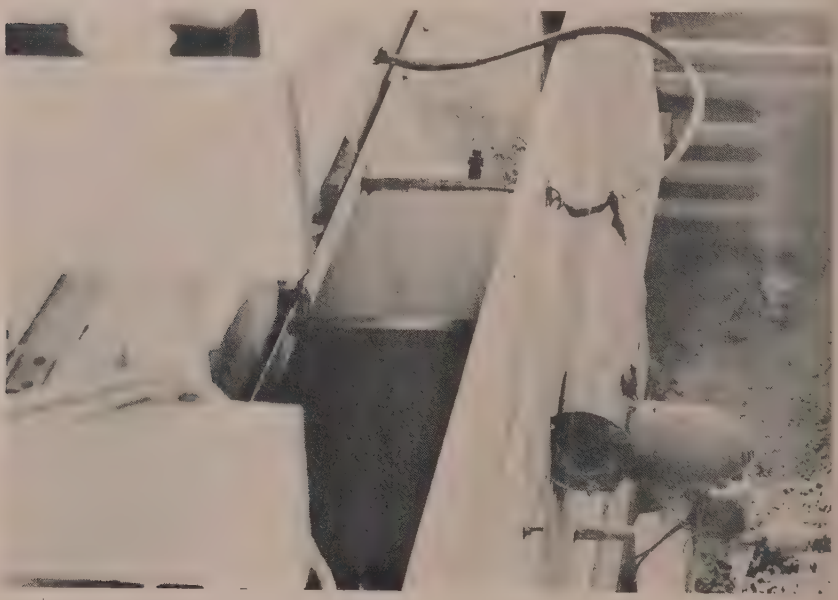

Fig. 6. Rear view of the controlled environment apparatus shows means of air exhaust and the solenoid valve controlling the water source. The solenoid is regulated by the humidostat and thermostat to control humidity and air temperature.

relay was wired in parallel with the humidostat and located in the front end of the air-exhaust box. When temperatures were higher than desirable, the temperature relay kept the mist nozzles and exhaust fan running even though the relative humidity was adequate. The propagating frame was thus cooled by evaporating water on the burlap covered forms at each end. Using this device, temperatures could be maintained at 75°F. ± 2°, even on the hottest summer days at the Irrigation Experiment Station, Prosser, Washington. Relative humidity could be maintained at 95 per cent or above when under illumination 14 hours a day. Illumination was supplied to encompass the dark hours, 6 P.M. to 8 A.M. By this means the ambient heat developed via illumination prevented a drop in the cutting frame temperature below 75°F. during the night.

Eight, 15.5″ x 17″ x 3″ flats were constructed from cedar. Each was filled with a mixture of two-thirds coarse, screened sand and one-third Canadian peat moss.

Four such flats were placed on both sides of the center vent so that air movement would not be obstructed. Flats were elevated one-half inch above the floor chamber on ½-inch square stringers. This system allowed good drainage and better aeration of the medium. Use of flats also facilitated handling and limited any spread of pathogenic organisms which might get started in the medium. In any one experiment a particular flat, therefore, could be used as a replication in a randomized design.

Standard treatment of cuttings

Except as indicated in specific experiments, the materials and treatments listed below were used on cuttings.

A solution was made of the following materials in the concentrations indicated, and will be referred to as the antitranspirant-nutrient solution:

Solution	*Strength*
Wax-based antitranspirant (See formula page 10)	1 to 30 parts water
Sucrose	5000 ppm
Urea	1000 ppm
Thiamine	25 ppm
Nicotinic acid	25 ppm

A second solution was made of two-hundred parts per million adenine dissolved in distilled water. It was mixed the day before use, since adenine dissolves very slowly in water.

Cuttings were immersed in the antitranspirant-nutrient solution momentarily, being sure that all the surface of the cutting was covered by the solution, and then laid on a porcelain sheet to air-dry. If adenine was to be used in treatments, cuttings were separated into groups and placed in 600 ml. beakers. The bases of the cuttings were immersed about one-half inch, either in distilled water, or in the adenine solution. Cuttings were removed after soaking for three hours.

Cuttings to be treated with the growth regulator were dipped in Hormodin No. 2 powder (3000 ppm. indolebutyric acid in talc). One-half inch of the base of the cutting was covered with the material. Cuttings were tapped against the edge of the container to remove excess regulator and set in the rooting medium. Cuttings not treated with growth regulator powder were set in the rooting medium directly.

A fine trickle of tap water was used to pack the medium firmly about the bases of the cuttings after benching. Then flats were placed in the controlled environment chamber. Results on rooting tendencies were taken approximately three weeks after the treatment, or as otherwise indicated.

Rooting Experiments

Interaction of nutrient and treatment combinations on the rooting of P. mahaleb

A factorial experiment was designed to measure the individual effects and interactions of the selected nutrient materials and metabolites in the antitranspirant-nutrient solution in combination with adenine, growth regulator, or growth regulator plus adenine.

Special procedures

One liter of the diluted antitranspirant material, plus one of the following combinations of materials, were placed in stone crocks.

Material combination	*Symbol*
Check (antitranspirant alone)	(1)
Sucrose (5000 ppm.)	S
Urea (1000 ppm.)	U
Sucrose and urea	SU
Thiamine (25 ppm.)	T
Sucrose and thiamine	ST
Urea and thiamine	UT
Sucrose, urea and thiamine	SUT
Nicotinic acid (25 ppm.)	N
Sucrose and nicotinic acid	SN
Urea and nicotinic acid	UN
Thiamine and nicotinic acid	TN
Sucrose, thiamine and nicotinic acid	STN
Urea, thiamine and nicotinic acid	UTN
Sucrose, urea, thiamine and nicotinic acid	SUTN

Thirteen hundred and fifty cuttings were made on July 1 from a *P. mahaleb* tree registered as MH-907 by the Department of Plant Pathology in cooperation with the State of Washington Nursery Improvement Program. Cuttings were separated into fifteen groups of ninety cuttings each, and immersed in the respective solutions, dried, and then separated into three sub-groups of thirty cuttings each.

The bases of cuttings of the first sub-group were immersed one-half inch in distilled water. The bases of cuttings of the second and third sub-groups were immersed one-half inch in 200 ppm. adenine solution. Cuttings were allowed to soak for three hours, after which they were removed. Then the bases of sub-groups one and two were dipped in Hormodin No. 2 powder. Sub-group three was given no further treatment.

Owing to the number of cuttings and treatments that had to fit into the shape and size of the controlled environment chamber, there was no alternative but to use a randomized design. Therefore, a complete randomization was used, modified in so far as to prevent more than one treatment from being replicated twice in one flat. Each sub-group was separated into three sets of ten cuttings each; *i.e.,* treatments in triplicate were coded and assigned to one of the eight flats at random, observing the stratification mentioned.

Results

Only a few, erratically distributed cuttings rooted without growth regulator; therefore, data from the use of adenine without IBA are not presented. A table of treatment means (Table 1) is presented on the basis outlined by Cochran and Cox (18). The effects measured were the percentage rooting, the average number of roots per cutting, and the percentage of cuttings breaking bud dormancy.

Table 1. Table of treatment means in the rooting response of *Prunus mahaleb* to all combinations of sucrose, urea, thiamine, nicotinic acid and adenine in conjunction with indolebutyric acid.

Treatment	Mean percentage rooting[a]	Mean number of roots per rooted cutting[a]	Mean percentage cuttings breaking bud dormancy[a]
(1) (IBA alone)	10.0	3.00	3.3
S (Sucrose)	26.7	4.25	10.0
U (Urea)	33.3	4.20	50.0
SU	13.3	5.00	43.3
T (Thiamine)	43.3	9.23	20.0
ST	16.7	4.20	16.7
UT	20.0	6.67	56.7
SUT	33.3	4.90	73.3
N (Nicotinic Acid)	43.3	5.08	13.3
SN	16.7	4.83	10.0
UN	6.7	3.00	20.0
SUN[b]	31.0	.....	-----
TN	30.0	3.77	16.7
STN	26.7	5.37	20.0
UTN	43.3	5.31	20.0
SUTN	50.0	5.67	70.0
A (Adenine)	16.7	2.20	3.3
SA	36.7	5.09	13.3
UA	30.0	4.22	36.7
SUA	43.3	6.08	66.7
TA	50.0	7.40	36.7
STA	33.3	5.90	26.7
UTA	50.0	5.67	63.3
SUTA	43.3	5.85	73.3
NA	36.7	5.91	10.0
SNA	23.3	5.00	20.0
UNA	13.3	2.50	26.7
SUNA[b]	44.7	-----	-----
TNA	40.0	6.67	33.3
STNA	36.7	7.27	36.7
UTNA	63.3	6.52	60.0
SUTNA	76.7	6.52	73.3

[a] Based on 30 cuttings (10 cuttings in 3 sets).
[b] Adjusted value obtained from subsequent data.

Table 2. Mean effects or interaction in rooting response of *P. mahaleb* cuttings to sucrose, urea, thiamine, nicotinic acid and adenine in all combinations.

Treaments	Sums	1	2	3	4	5	Mean effects or interaction	
(*1*) (IBA alone)	30	110	250	590	1343	3257	(Mean)	
s	80	140	340	753	1914	77	S	1.604
u	100	180	303	910	—37	317	U	6.604**
su	40	160	450	1004	114	377	SU	7.854**
t	130	190	380	—50	43	683	T	14.229**
st	50	113	530	13	274	—217	ST	—4.520
ut	60	170	354	30	183	303	UT	6.313*
sut	100	280	650	84	194	83	SUT	1.729
n	130	160	—10	10	237	257	N	5.354*
sn	60	220	—40	33	446	117	SN	2.438
un	20	250	3	90	—23	117	UN	2.438
sun	93[a]	280	10	184	—194	337	SUN	7.021**
tn	90	180	100	10	137	203	TN	4.229
stn	80	174	—70	173	166	183	STN	3.813
utn	130	230	54	10	117	463	UTN	9.646**
sutn	150	420	30	184	—34	—477	SUTN	—9.938**
a	50	50	30	90	163	571	A	11.896**
sa	110	—60	—20	147	94	157	SA	3.271
ua	90	—80	—77	150	63	231	UA	4.813*
sua	130	40	110	296	54	11	SUA	0.229
ta	150	—70	60	—30	23	209	TA	4.354
sta	100	73	30	7	94	—171	STA	—3.563
uta	150	—10	—6	—170	163	29	UTA	0.604
suta	130	20	190	—24	174	—151	SUTA	—3.146
na	110	60	—110	—50	57	—69	NA	—1.438
sna	70	40	120	187	146	—9	SNA	—0.188
una	40	—50	143	—30	37	71	UNA	1.479
suna	134[a]	—20	30	196	146	11	SUNA	0.229
tna	120	—40	—20	230	237	89	TNA	1.854
stna	110	94	30	—113	226	109	STNA	2.270
utna	190	—10	134	50	—343	—11	UTNA	—0.229
sutna	230	40	50	—84	—134	209	SUTNA	4.354

Standard error 2.386
*L.S.D. .05 ..... 4.772
**L.S.D. .01 ..... 6.346
[a] Adjusted value obtained from subsequent data.

The treatment combination, sucrose-urea-nicotinic acid with and without adenine (SUN and SUNA) were not included in the initial experiment. These treatments have been inserted as adjusted values from subsequent tests. Data on mean number of roots per cutting and mean percentage cuttings breaking bud dormancy were not obtained for these subsequent treatment combinations. Because of these missing data only interaction for mean percentage rooting is evaluated. The mean effects or interaction between responses and chemical combinations are presented in Table 2 according to the procedures outlined by Yates (70).

It is apparent from this analysis that considerable interaction has occurred, as brought out statistically. The mean effects or interactions appear erratic and of vague meaning when reference is made to the actual rooting results obtained from the basic data. However, it must be remembered that the data on a specific combination of materials are weighed against all other combinations containing that specific combination or any of its constituents. Hence, additive and subtractive influences of all sets of combinations containing the given treatment or any of its constituents show up in the mean effects for the given treatment. They are expressed as non-significant, significant or highly significant.

As the combinations of materials increased in number, the complexity of interactions increased. Thus, when we look at the combination SUTN, at first it might appear difficult to comprehend why we have a highly significant minus effect. This value is such because the rooting response should have been much higher than the data show. It should have been higher because rooting responses of certain combinations that can be made of SUTN materials point that out.

This is determined by weighing and counter-weighing all the pertinent combinations incorporated in the SUTN formulation. In other words the rooting response of SUTN should have been above a value of 200. Hence, a very great depressing response is apparent in the combination SUTN which perhaps is caused by some limiting factor or factors not disclosed in the relative data being interpreted.

If we look at the combination SUTNA we are aware that adenine, perhaps in part is a limiting factor. Or, perhaps it acts as a substitute to nullify the minus effect produced by a limiting factor or factors. True, the mean effects for SUTNA are not significant, *per se.* The tremendous negative value of SUTN had to be overcome. The non-significance of SUTNA means that some other factor or factors which this study does not include is limiting, or the amount of one of the factors being considered became limiting.

A little closer appraisal indicates the probable limiting response. That is, the combination of S and T are antagonistic in that it is observed that their mean effects are negative or small.

Although the combination of S and T is the probable limiting agent, there may be other unknown limiting agents involved. Specific concentrations of ingredients were used in this study. This is additional uncontrolled error, since the balance between known factors and any unknown reactant factor or factors is not measured.

The comparisons of nicotinic acid and thiamine, members of the Vitamin B complex, are interesting. As already noted, thiamine and sucrose appear to be antagonistic, while that impression isn't so great with nicotinic acid and sucrose. The effect of nicotinic acid is not significant in the presence of urea, but the response to thiamine with urea is almost highly significant. The effect of thiamine with nicotinic acid is not significant, however. The interaction effect of thiamine and nicotinic acid with urea is very

highly significant. Thiamine and nicotinic acid with sucrose is not a significant effect, while sucrose and urea is highly significant. Sucrose and urea with nicotinic acid still produces a highly significant interacting effect, but the corresponding effect of sucrose and urea with thiamine is not significant. Thus, the mechanism for rooting responses attributable to nicotinic acid seems different from that of thiamine.

The whole underlying aspects of the experiment denote a complex nitrogen utilization and an indistinct metabolism of the element in relation to Vitamin B members.

The increase in the average number of roots per rooted cutting (Table 1) seems to be directly related to thiamine. Considerable interaction is also indicated, but unfortunately indeterminable because of missing data. No other material approached the ability of thiamine to induce abundant numbers of roots per rooted cutting. However, it is not believed that the increase in numbers of roots per rooted cutting compensates for the higher percentages of rooting obtained when the other materials are also used in the treatment. Here again it is obvious that some unknown limiting factor or factors is responsible for lack of the ultimate response that should have been obtained.

Of more importance in a practical sense than the number of roots per rooted cutting, perhaps, is the problem of breaking bud dormancy so that rooted cuttings can become established. It is evident from Table 1 that urea was the primary factor in the breaking of bud dormancy. Treatments containing urea, sucrose and thiamine, induced the maximum response. Nicotinic acid tends to be a depressant in the breaking of bud dormancy.

Effect of the apical bud on rooting responses of a P. mahaleb *tree difficult to root*

One of the progeny of the *P. mahaleb* tree registered as MH-907 by the State of Washington Nursery Improvement Program was brought from Prosser to the greenhouses in Pullman. Preliminary tests had indicated that it would not root when treated with Hormodin No. 2 powder unless the apical bud was present on a cutting.

Special procedures

One hundred and twenty cuttings from mature shoots were made on March 14 from this seedling. Forty-five were made several inches below the terminal end of the shoot and seventy-five were made from the terminal end of the shoot.

The apical bud was removed from fifteen of these terminal cuttings. Five of these disbudded terminal cuttings were added to each of three groups of fifteen cuttings made from the non-terminal regions of shoots. These three groups of twenty cuttings were classed as "non-terminal" cuttings because all lacked an apical bud.

The remaining sixty cuttings, which had an apical bud, were separated into three groups of twenty cuttings each and classed as "terminal" cuttings.

Cuttings were processed as indicated in standard procedures (page 13) so that one group in each class was treated with adenine, one with growth regulator, and one with adenine plus growth regulator.

Table 3. The effect of the apical bud on the rooting response of a *P. mahaleb* selection to adenine and indolebutyric acid.

Type of cutting	Treatment		
	3000 ppm. IBA	200 ppm. adenine plus 3000 ppm. IBA	200 ppm. adenine
With apical bud	70[a]	70	0
Without an apical bud	0	80	0

[a]Percentage rooting based on 20 cuttings.

Results

The percentage rooting response for each treatment is in Table 3. Typical cuttings are shown in figure 7. Groups treated with adenine alone did not root, and are not pictured.

These data indicate that cuttings of *P. mahaleb* without an apical bud root well when treated with adenine plus indolebutyric acid at the concentrations used. No rooting occurred on such "non-terminal" cuttings treated with IBA alone, or treated with adenine alone. It appears, therefore, that a ratio between the two materials may be contingent to rooting. Perhaps vascular differentiation of meristematic tissue at the base of the cutting into roots by IBA, is augmented by adenine. Shoot-tip cuttings whose apical buds had been removed responded similarly to those cuttings made from lower regions of the shoot.

The results obtained on the "terminal" cuttings bearing an apical bud also support the suggestion of a ratio between IBA and adenine. In contrast to "non-terminal" cuttings (lacking an apical bud), IBA influenced initiation of roots on "terminal" cuttings (having an apical bud). Therefore, the apical bud may be associated with the production and translocation of adenine or a comparable material. A comparison of the degree of rooting response shows a difference in the length and numbers of roots produced on the two classes of cuttings (see figure 7). "Non-terminal" cuttings treated with adenine and IBA produced more and longer roots than "terminal" cuttings treated with these two materials. Furthermore, "terminal" cuttings treated with adenine and IBA had fewer and shorter roots in general than "terminal" cuttings treated with IBA alone. This result seems to indicate that treatment of "terminal" cuttings with both materials caused an "unbalance" between IBA and adenine. However, this conclusion postulates that the adenine augmented materials are indigenous to the apical bud.

If this unknown factor of the apical bud is quite similar to adenine or is a different material, the possibility of antagonism might be indicated. In any event, it is quite apparent that cuttings lacking an apical bud which were taken from the *P. mahaleb* tree being tested, though "hard-to-root" as compared with most *P. mahaleb* individuals, could be rooted when treated with adenine and IBA.

Fig. 7. The effect of an apical bud on the rooting of cuttings of a *P. mahaleb* selection, and substitution of the effect by treatment with indolebutyric acid and adenine. Upper left: cuttings with an apical bud treated with indolebutyric acid. Upper right: cuttings with an apical bud treated with adenine and indolebutyric acid. Lower left: cuttings without an apical bud treated with indolebutyric acid. Lower right: cuttings without an apical bud treated with indolebutyric acid and adenine. Cuttings treated with adenine alone did not root and are not shown.

Rooting variability of a P. mahaleb *selection in relation to maturity and treatment*

A particular *P. mahaleb* introduction from the Near East (P. I. 174329), having excellent horticultural features and testing free of known viruses, has been investigated in some detail. Rooting responses associated with age of shoots were investigated on one individual from this introduction.

Special procedures

Shoot maturity was judged on an arbitrary classification of woodiness in conjunction with the development of an apical bud. "Succulent" cuttings were those made from shoots which had set no apical bud, and which would not break when bent double. "Slightly-woody" cuttings were those from shoots which had set an apical bud, but which would not break when bent double. "Semi-woody" cuttings were from shoots which had set an apical bud and which would break in two when the shoot was bent double. "Woody" cuttings were well-matured

shoots which would fracture but would not snap in two when bent double.

Tests were made on March 2, April 21, May 15, June 9, July 6, August 31, and September 28. Cutting material used in tests on March 2, April 21, May 15, and June 9 was taken from year-old trees grown from rooted cuttings of the selected individual. The rooted trees had been grown through the winter in the greenhouse.

Shoots were very "woody" when cuttings were made in March, but new, "succulent" growth was used in April. The shoot growth had become "slightly-woody" by the time cuttings were made in May and was "semi-woody" in June. Cutting material used in tests on July 6, August 31, and September 28 was taken from the parent tree growing in the Plant Pathology "Scion Block" at Prosser, Washington. July cuttings were made from "slightly-woody" shoots; cuttings made in August and September were "woody." All cuttings were made from middle portions of shoots.

Cuttings were treated as indicated in Standard Treatment of Cuttings (page 13) and were evaluated three weeks after treatment for:

1. percentage of cuttings rooted
2. number of roots per cutting
3. length of roots produced.

Results

The results obtained in these tests are in Table 4. It appears that rooting response varied with the degree of maturity of cuttings treated with growth regulator. On "succulent" cuttings, adenine increased rooting. As tissues became more "woody," growth regulator alone increased the rooting response to a level where adenine had no apparent value. However, a comparison of the average number of roots per cutting, and the average length of the roots produced, indicates that cuttings treated with adenine had more and longer roots.

The evidence is conclusive that:

1. "Semi-woody" to "woody" cuttings of this selection root well when treated with growth regulator alone.
2. More "succulent" cuttings that do not respond to treatment with growth regulator alone will root if treated with adenine plus growth regulator.

Effect of adenine in determining the site of root initiation on a particular P. mahaleb *selection*

A particular seedling which was one of the progeny of the *P. mahaleb* tree registered as MH-907 was observed to develop roots from the side of the cutting stem above basal callus formation. This action was very seldom witnessed on other individuals treated with Hormodin No. 2 powder. It was noted that cuttings of this selection treated with adenine plus Hormodin No. 2 powder produced only basal roots. The selection rooted very satisfactorily after treatment with the growth regulator alone, and adenine had no apparent influence on percentage of cuttings rooting. A test of the ability of adenine to determine the site of root formation was made, using cuttings made from greenhouse forced shoots that were very "woody."

Special procedures

Sixty cuttings were made and separated into three groups of twenty cuttings each. Two groups were immersed in the antitranspirant-nutrient solution. The third group was immersed in antitranspirant-nutrient solution to which adenine had

Table 4. The effect of shoot maturity on the rooting response of *P. mahaleb* to treatment with indolebutyric acid or indolebutyric acid and adenine.

	Greenhouse material								Field material					
	March 2		April 21		May 15		June 9		July 6		August 31		September 28	
	IBA	IBA plus adenine	IBA	IBA plus adenine	IBA	IBA plus adenine	IBA	IBA plus adenine	IBA	IBA plus adenine	IBA	IBA plus adenine	IBA	IBA plus adenine
Percentage cuttings rooted	70	75	30	70	65	85	80	100	68	87	92	90	90	80
Average No. roots per cutting	5.8	8.1	3.7	6.2	5.3	4.5	9.4	10.7	11.0	12.4	10.6	11.8	10.2	9.8
Average length of roots in inches	.82	.77	.86	.84	1.01	1.79	.94	1.33	.91	1.40	.87	.93	.81	1.07
Condition of tissue	"woody"		"succulent"		"slightly woody"		"semi-woody"		"slightly woody"		"woody"		"woody"	
No. of cuttings per treatment	20		10		20		20		60		50		20	

been added to make a 200 ppm. concentration. Bases of cuttings of the latter group and one of the former groups were treated with Hormodin No. 2 powder. The remaining group was treated with Hormodin No. 2 to which adenine sulphate had been added to make a concentration of 650 ppm.

Results

The results obtained are in Table 5. Adenine had a marked effect on the site of root formation on cuttings. Morphologic examinations were not made, but it was noted that roots produced above the bases of the cuttings ruptured the bark and internal callus. Extensive production of callus tissue at the base of cuttings was also apparent. When roots were formed at the bases of cuttings, callus tissue did not rupture. Roots appeared to rise from the callus tissue and not adjacent to it.

The number of roots produced, and the average length of roots agreed with the results of other tests involving adenine and IBA on the rooting of *P. mahaleb*. Cuttings treated with adenine produced more and longer roots per cutting.

Table 5. The site of root formation on cuttings of a *P. mahaleb* selection and other rooting responses as influenced by adenine and indolebutyric acid.

	Antitranspirant-nutrient solution containing 200 ppm. adenine	Antitranspirant-nutrient solution *not* containing adenine	
	IBA	IBA	IBA plus 650 ppm. adenine sulphate
Percentage of cuttings rooted[a]	60	80	80
Average number of roots per cutting	12.5	10.8	13.5
Average length of roots in inches	.61	.54	.79
Percentage of cuttings rooted above base[a]	5	65	0

[a] Based on 20 cuttings per treatment.

Effect of adenine and re-treatment with growth regulator on the rooting responses of a P. avium *selection*

Prunus avium, the Mazzard cherry, is considered a "hard-to-root" species. "Hard-to-root" species of other woody plants have been shown to root well if re-treated with growth regulator (Deuber, 21; Warner and Went, 60). Therefore, tests were conducted on propagation of the Mazzard cherry, using this procedure in combination with adenine.

The source of cutting material was rootstock suckers of a greenhouse forced Shirofugen flowering cherry. Preliminary

tests almost exactly a year previous to those considered below were negative. In those preliminary tests cuttings were given a single treatment of Hormodin No. 2 powder (3000 ppm. in talc). Extensive callus formed, but no rooting had occurred after 8 weeks.

Special procedures

Thirty cuttings made from shoots arising from the rootstock mentioned above were treated as indicated in Standard Treatment of Cuttings (page 13). Ten cuttings received antitranspirant-nutrient solution plus adenine, ten cuttings received antitranspirant-nutrient solution plus Hormodin No. 2 powder, and ten cuttings received antitranspirant-nutrient solution plus adenine and Hormodin No. 2 powder.

The experiment was begun April 14. Three weeks later (May 4) all cuttings were re-treated with Hormodin No. 2 powder. Two weeks after this (May 18) all unrooted cuttings were again re-treated with Hormodin No. 2 powder. Final readings were made on June 8, three weeks after the last treatment.

Results

The results of this study are in Table 6. It is apparent that adenine retarded root production. Re-treated cuttings receiving growth regulator alone rooted satisfactorily five weeks after the initial treatment. Re-treatment of unrooted cuttings with growth regulator at this time induced rooting of all cuttings three weeks later (the eighth week).

In contrast, cuttings initially treated with adenine plus growth regulator, and re-treated in three weeks with growth regulator, rooted only slightly by the fifth week. However, when unrooted cuttings of this group were again re-treated with growth regulator, all cuttings had rooted by the eighth week. Apparently, the retarding effect of adenine had been overcome.

The cuttings treated first with adenine alone, and re-treated three weeks later with growth regulator, failed to root by the end of the fifth week. Additional re-treatment of cuttings with the growth regulator resulted in very limited rooting by the end of the eighth week.

Further re-treatment of the unrooted cuttings of this group might have induced a high percentage of rooted cuttings. In all cases where adenine was used, fewer roots were produced.

Two things were common in all three groups:

1. No cuttings rooted until five weeks after the initial treatment with indolebutyric acid.
2. At least one re-treatment of cuttings (with IBA) was necessary to induce rooting.

Table 6. The effect of adenine and re-treatment with Hormodin No. 2 Powder (IBA) on the rooting response of a *P. avium* selection.

Initial Treatment	Accumulated percentage rooting[a]		
	Re-treatment[b]		Final reading
April 14	May 4	May 18	June 8
IBA	0	70	100
IBA plus 200 ppm adenine	0	20	100
200 ppm adenine	0	0	10

[a] Based on 10 cuttings per treatment.
[b] All unrooted cuttings were re-treated with Hormodin No. 2 powder (3000 ppm. IBA in talc).

The re-treatment of cuttings with IBA indicates a possible need for an extended supply of growth regulator. But subsequent tests using lanolin as a carrier to extend the period of growth regulator supply did not induce satisfactory rooting of *P. avium.*

The possibility that the 3000 ppm. concentration of growth regulator in talc was not high enough was investigated. Single applications of growth regulator up to concentrations of 8000 ppm. in talc gave negative results in all tests. Frequently, injury occurred at higher concentrations of the regulator.

Rooting responses of a P. persica *after treatment with adenine*

In all preliminary tests on the rooting of *P. persica* selections, cuttings treated with adenine plus growth regulator produced fewer and shorter roots than similar cuttings treated with growth regulator alone. There appeared to be no material difference in the percentage of cuttings rooting, however. A test was run to measure the amount of suppression of root formation and growth, since such a response was in contrast with results obtained on *P. mahaleb* selections being tested.

Special procedures

Thirty cuttings were made from a greenhouse-grown seedling of the Lovell peach variety, and separated into three groups of ten cuttings each. Two groups were immersed in the antitranspirant-nutrient solution. The third group was immersed in antitranspirant-nutrient solution to which enough adenine had been added to make a concentration of 200 ppm. Cuttings of one of the former two groups and the latter group were then treated with Hormodin No. 2 powder. The remaining group was treated with Hormodin No. 2 powder to which adenine sulphate had been added to make a concentration of 650 ppm.

Results

Results of this test are in Table 7. Apparently, adenine had little effect on the percentage of cuttings rooting, but it did strongly inhibit the number and length of roots produced. Cuttings treated with adenine produced extensive basal callus, in marked contrast to results obtained on other species of *Prunus* tested.

Table 7. The response of *P. persica* to treatment with indolebutyric acid and adenine.

Treatment	Percentage rooting[a]	Av. no. roots per cutting	Av. length of roots
Hormodin No. 2	70	20.16	1.12
200 ppm. adenine as a foliar dip and Hormodin No. 2	60	10.40	.33
Hormodin No. 2 containing 650 ppm. adenine sulphate	80	6.25	.34

[a] Based on 10 cuttings per treatment.

Rooting responses of P. cerasifera *var.* myrobalan

It is customary in nursery practice to propagate *P. cerasifera* var. *myrobalan* from dormant cuttings. However, softwood cuttings were used in the present study to be consistent in the type of cutting material used for rooting responses of other *Prunus* spp. Cutting material was taken from a tree registered as MY-113 by the State of Washington Nursery Improvement Program.

Effect of growth habit and apical bud on rooting

Considerable variation in rooting of untreated checks was noted in preliminary tests. Mr. H. Manten of the Manten Nursery in White Rock, B. C., had observed that rooting of this species from softwood cuttings depended upon where cuttings were taken from the tree. This report indicated the need for a position test to compare the rooting response of vertical *vs.* horizontal shoot growth.

Special Procedures.—On July 20 thirty cuttings were made from shoots growing vertically and thirty cuttings were made from shoots growing horizontally. Twenty of the cuttings from each group did not possess an apical bud; 10 cuttings from each did have apical buds. Cuttings were set in a medium composed of two-thirds coarse pit-sand and one-third Canadian peat moss. Standard propagating box procedures were employed; cuttings were syringed in the morning and evening. Readings were made four weeks after the experiment began.

Results.—The results obtained are in Table 8. Untreated cuttings with an apical bud rooted well, regardless of growth habit. Cuttings made from horizontally growing shoots without an apical bud rooted well. Only one cutting made from vertically growing shoots without an apical bud rooted.

This result indicates that the apical bud may control some inhibiting influence on root formation which is not present in horizontally growing shoots. Another possibility is that only the apical bud of vertically growing shoots possesses enough of some material, probably a natural auxin, to offset a geotropic movement of this material out of the stem.

Rooting in relation to growth habit after treatment with growth regulator and adenine

An experiment was set up to note the rooting of *P. cerasifera* var. *myrobalan* cuttings in relation to habit of growth and treatment with growth regulator and adenine. The same source tree (MY-113) that was tested in the preceding experiment was used.

Special Procedures.—Eighty cuttings were made from vertically growing shoots and eighty cuttings were taken from horizontally growing shoots. Growth was

Table 8. Effect of habit of growth on the rooting of a *P. cerasifera* var. *myrobalan* selection.

	Vertical shoot growth		Horizontal shoot growth	
	With an apical bud	Without an apical bud	With an apical bud	Without an apical bud
Percentage rooting[a]	100	5	90	85

[a] Based on 10 cuttings with an apical bud and 20 cuttings without an apical bud.

somewhat "succulent"; therefore, the terminal eight inches of the shoots were discarded before the cuttings were made. Each group was separated into four lots of twenty cuttings each.

All lots were treated with the antitranspirant-nutrient solution as indicated in Standard Treatment of Cuttings (page 13). Two lots from each group were treated with 200 ppm. adenine solution for one and one-half hours. The remaining two groups were treated for a similar period with distilled water.

Then, of the two lots from each group receiving adenine, one was treated with Hormodin No. 2 powder while the other was given no further treatment. One of the two remaining lots from each group was treated with Hormodin No. 2 powder, while the other was given no further treatment.

Results.—The results obtained are in Table 9. The data indicate that adenine definitely inhibits root formation in this species. The results parallel those on *P. avium* and *P. persica.* Apparently treatment with growth regulator eliminated the habit of growth effect, and adenine is an antagonist of indolebutyric acid in this respect. It is also apparent that adenine completely destroyed the habit of growth effect.

Table 9. The rooting of *P. cerasifera* var. *myrobalan* cuttings without an apical bud in relation to growth regulator, adenine, and to habit of growth.

Growth habit	Treatment[a]			
	Adenine	Adenine plus IBA	IBA	Check
Percentage rooting from vertical habit of growth	0	70	100	25
Percentage rooting from horizontal habit of growth	0	65	100	95

[a] Based on 20 cuttings per treatment.

Discussion and Conclusions

Certain special procedures used to overcome some of the difficulties in the rooting of *Prunus* spp. cuttings can be used commercially. The results show that an antitranspirant greatly benefits vegetative propagation by cuttings. The antitranspirant can be applied in two phases. First, it can be used to limit transpiration from cuttings prior to rooting. Second, it can be used on the newly produced foliage of rooted cuttings just before transplanting. The latter use compensates for lack of cuticle formation brought about by high humidity in the propagating chamber.

The material is a cheap substitute for continuous-mist procedures used to control water loss from cuttings. In areas where water is hard, mist leaves damaging salt deposits on foliage. Using an antitranspirant avoids the salt problem. The antitranspirant used is also a satisfactory carrier of nutrient materials to be absorbed through the foliage. The application of nutrients on the foliage of cuttings is a new practice in vegetative

propagation and could also benefit the nursery industry. The procedure is simple, inexpensive, and requires no special equipment.

The different physiological reactions among *Prunus* spp. brought about by the same chemical materials emphasize the different rooting requirements among species of the genus. In *P. mahaleb,* considerable variation occurs even below the species level. Although certain general statements can be made about rooting responses of a particular species, it is evident that certain individuals within a species may need special consideration to obtain the best results.

The variation among individuals within a *Prunus* sp. was so marked in certain tests where conditions and treatments were the same, that the conclusions of Gardner (24) in regard to age of tree and rooting response are questionable. He compared one-year-old seedlings with two-year-old seedlings, not taking into account the differences in genotypes. Admittedly, there was a tendency for all *Prunus* spp. tested, except *P. persica,* to root better when cuttings were made from younger trees. But it is believed that different results would have been obtained if clonal material had been used.

Treating succulent cuttings from *P. mahaleb* selections with adenine seemed to compensate for lack of cutting maturity. This result undoubtedly is due to differences in the physiological states of cuttings whose tissues differ in maturity. Other species did not respond to treatment with adenine, indicating that adenine may be a substitute for some organic compound used in the metabolism of *P. mahaleb.* The retarding or inhibiting effect of adenine on the other species suggests an antagonism between adenine and some naturally occurring substance or substances, or perhaps an increase in adenine to a toxic level.

It is possible to visualize a difference between the gene complement of *P. mahaleb* and other *Prunus* spp. which would allow use of adenine by an alternate metabolic pathway. This explanation includes an assumption that other *Prunus* spp. do not possess a particular gene or group of genes to allow this pathway, or that they have a gene or group of genes which is associated with the production of some material that blocks the pathway by antagonism.

Except for the response of *P. mahaleb* to treatment with adenine, all other *Prunus* spp. selections tested seem to support the results obtained by Skoog and Tsui (57). They showed that adenine is associated with the control of growth regulator activity. Tests such as paper chromatographic techniques should be undertaken. Purine levels in tissues of *Prunus* spp. should be studied with respect to maturity of shoot growth and rooting tendencies. Such a method might allow one to determine the most advantageous concentration of growth regulator to use at a particular time, if a balance between growth regulator and adenine or some other purine material is a primary factor in root formation.

From the results obtained in these studies it is concluded that:

1. *Prunus* spp. vary individually in response to natural or induced rooting of softwood cuttings.
2. *Prunus mahaleb* cuttings can be propagated any time during the growing season if treated with suitable nutrients in an antitranspirant carrier plus indolebutyric acid and adenine.
3. Adenine compensates for lack of maturity in the rooting of certain *P. mahaleb* cuttings.
4. Adenine substitutes for a naturally occurring substance in *P. mahaleb* associated with root formation. This substance is controlled or produced by the apical buds of certain *P. mahaleb* individuals.
5. Adenine may determine the site of

root formation on certain *P. mahaleb* individuals.

6. Certain selections of *Prunus avium* can be rooted satisfactorily when retreated with 3000 ppm. indolebutyric acid in talc.
7. Softwood cuttings of *Prunus persica* and *Prunus cerasifera* var. *myrobalan* root very satisfactorily when treated with an antitranspirant solution containing nutrients and then with 3000 ppm. indolebutyric acid in talc.
8. Adenine decreases or inhibits the rooting of *P. persica, P. avium* and *P. cerasifera* var. *myrobalan* softwood cuttings which have been treated with growth regulator.
9. Certain untreated *P. cerasifera* var. *myrobalan* softwood cuttings without an apical bud root well when made from horizontally growing shoots, but do not root when made from vertically growing shoots.
10. Indolebutyric acid induces maximum rooting of *P. cerasifera* var. *myrobalan* softwood cuttings, regardless of growth habit.
11. The difference in the rooting of *P. cerasifera* var. *myrobalan* cuttings associated with growth habit is due to lack of movement of naturally occurring substances out of horizontal shoots.
12. Except for *P. mahaleb,* adenine appears to be associated with control of growth regulator activity in all *Prunus* spp. tested.

Summary

The factors for successful vegetative propagation of *Prunus* spp. lie in two categories:

1. environmental control of the propagating chamber.
2. physiological conditions inherent in, or imposed upon the cuttings, both before and during rooting.

Softwood cuttings of *Prunus* spp. having two or three leaves with a smooth basal cut just below a bud, rooted very satisfactorily when treated with certain chemicals.

An automatically regulated environment chamber was constructed to control light, temperature, and humidity, using forced ventilation, mist spray, and fluorescent lamps, augmented by incandescent bulbs.

A paraffin emulsion (antitranspirant) diluted one part to thirty parts water and used as a foliar dip, reduced desiccation of *Prunus* cuttings until roots were produced.

Varying responses were obtained from foliar applications of 5000 ppm sucrose, 1000 ppm urea, 25 ppm thiamine, and 25 ppm nicotinic acid to cuttings. These materials were tested in all possible combinations placed in a paraffin emulsion (antitranspirant solution):

1. Combined with Hormodin No. 2 powder (3000 ppm IBA in talc) applied to bases of cuttings.
2. Combined with 200 ppm adenine in a soak of bases of cuttings for three hours.
3. Combined with both 1 and 2 above.

Little or no rooting occurred in the absence of growth regulator. With growth regulator all materials under test were either highly significant (thiamine, adenine and urea) or significant (nicotinic acid), except sucrose. However, in measuring the mean effects or interactions of all the various combinations of the materials in conjunction with growth regulator, limiting factors and/or antagonisms are apparent. Possible reasons for limited responses are discussed.

The greatest number of roots per rooted cutting was directly related to thia-

mine plus growth regulator. When thiamine was combined with any other of the materials, fewer roots per rooted cutting were produced.

The breaking of bud dormancy was directly related to nitrogen utilization, and hence, urea. There was no apparent connection between rooting tendencies of a particular treatment and the breaking of bud dormancy. When urea was combined with sucrose and thiamine, the greatest number of cuttings (73 per cent) broke bud dormancy. Nicotinic acid depressed the response.

An apical bud on softwood cuttings of *P. mahaleb* had a definite effect on the rooting of a certain "hard-to-root" individual. When given nutrients through the foliage, this selection rooted 70 per cent, if cuttings had an apical bud and were treated with Hormodin No. 2 powder. Similarly, 70 per cent of the cuttings that had an apical bud and were treated with Hormodin No. 2 powder plus adenine rooted, although fewer roots were produced.

Cuttings without an apical bud did not root when treated with Hormodin No. 2 powder alone. However, 80 per cent of such cuttings rooted when treated with indolebutyric acid and adenine; these cuttings grew more and longer roots than cuttings containing an apical bud. The effect on rooting owing to possible balance between adenine and auxin, and the relationship of these materials to the apical bud is discussed.

Response to treatment of *P. mahaleb* changed through the growing season. On succulent cuttings, adenine plus Hormodin No. 2 was significantly better as a rooting treatment than Hormodin No. 2 alone. As the tissue of cuttings became more woody, the growth regulator alone increased rooting until no difference was apparent between cuttings treated with the growth regulator and cuttings treated with the growth regulator plus adenine. However, the average number of roots per cutting and the average length of roots produced was greater when adenine was used with the growth regulator.

Adenine influenced the site of root initiation on one *P. mahaleb* selection. When treated with Hormodin No. 2 powder, cuttings from this individual produced roots above the base. When adenine was applied in combination with the growth regulator, either as a dip or mixed with the auxin, roots were almost entirely limited to the base of the cutting. Roots arose from the callus tissue produced on the cut surface. The number of roots per cutting and the average length of roots produced were greater when adenine was used with auxin.

Prunus avium can be induced to root satisfactorily (70 per cent) if re-treated with Hormodin No. 2 powder three weeks after initial treatment with the material. All unrooted cuttings again re-treated after the fifth week rooted by the end of the eighth week. The use of adenine with Hormodin No. 2 powder delayed rooting.

Use of nutrients on the foliage of *P. persica* softwood cuttings in combination with growth regulator permitted practical increase of this species at any time during the growing season. Adenine in conjunction with the growth regulator had no apparent effect on the percentage of rooted cuttings, but did markedly inhibit the average number of roots produced and the length of roots.

The growth habit of *P. cerasifera* var. *myrobalan* had a great effect on rooting of cuttings not treated with growth regulator.

Softwood cuttings made from vertically growing shoots rooted very little (5 per cent), unless the apical bud was present (100 per cent). Cuttings made from horizontally growing shoots rooted the same, whether the apical bud was present (90 per cent) or not (85 per cent).

However, cuttings treated with growth regulator responded differently. Regard-

less of the habit of shoot growth, indolebutyric acid caused 100 per cent rooting. Adenine, used alone, completely inhibited rooting, regardless of growth habit, and retarded rooting when growth regulator and adenine were used in combination.

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